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SOME FURTHER EXPERIMENTS ON SELF-FERTILIZATION IN CIONA.

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My previous experiments with *Ciona intestinalis* had shown that, by adding chloroform, or alcohol, or ammonia to the sea water, self-fertilization could sometimes be effected, although in this species self-fertilization does not usually take place in sea water. The experiments did not show why under normal conditions self-fertilization does not occur. Other results appeared to indicate that the eggs offer a resistance of some kind to the entrance of the spermatozoa of the same individual and that the lack of fertilization is due to the failure of the spermatozoa to enter, and not due to their failing to stimulate the eggs after entering. An analysis of the conditions led me to the conclusion that the inability of the spermatozoa to penetrate eggs of the same individual could not depend on differences in size of the micropyle of the egg that might be correllated, supposedly, with the size of the spermatozoa of the same individual; also that the failure was not due to the lack of some exciting substance present in eggs of other individuals. I hazarded the guess that the resistance offered by the egg to the entrance of its "own" sperm might be due to some substance contained in the egg, or in its membranes, that brings the spermatozoa of the same individual to rest, and I pointed out that this view could be tested by the following experiment. If the eggs of an individual (A) were removed and allowed to stand in sea water, and if then this water were poured off and the spermatozoa of the same individual (A) were added to the water, they should be so affected that they would fail to fertilize the eggs of another individual (B). I should have made the *proviso*, as subsequent events have shown, that

this result would follow, provided the substance that prevents self-fertilization is soluble in sea water. This experiment I have carried out during the past summer in a variety of ways, but have found that the anticipated results did not follow, from which I am led to conclude that the phenomenon is not due to a substance that is soluble.

The failure of this experiment to give positive results showed me that I must start once more at the very beginning, and test more thoroughly the assumption that the sperm of one individual, if in good condition, is capable of fertilizing equally well the eggs of all other individuals; for the difficulty of explaining the results becomes immensely greater if this condition holds absolutely. If it does hold it would mean that the conditions present in an individual are not found in any degree in any other individual. The principal results of this paper deal, therefore, mainly with this question, although, at the same time, I have described some other experiments which gave negative results, because, I think, the results, negative though they be, will be of value in determining the direction of further experiments.

My work was carried out at the marine laboratory of the University of California, situated at present at Coronado Beach, California. I am under many obligations to the university for the privilege of working at the station, and especially to the director, Professor William E. Ritter, and to the resident naturalist, Mr. B. M. Davis. It gives me great pleasure to acknowledge my appreciation of the many courtesies extended to me during my sojourn at the station. Professor Ritter informs me that the species of *Ciona* at Coronado Beach appears to be identical with *Ciona intestinalis* of Europe.

Experiments in Cross-fertilization.

The eggs of an individual were removed and distributed in six dishes, A–A. Similarly the eggs of another individual were taken out and distributed in six dishes, B–B; and, so on, for four other individuals. See Table I. The sperm of A was then removed from the vas deferens and added to A, B, C, D, E, F. In the table the sperm is indicated by the small letters, a–f, used as exponents. The sperm from each of the other five individuals was used in the same way. Thus all the eggs were

crossed with all the different kinds of sperm, and self-fertilization was also tried in each case. The self-fertilized lots form a diagonal line across the tables. In only a single case out of the many hundreds of eggs mixed with their own sperm did fertilization occur. It is needless, perhaps, to add that separate scissors, pipettes, etc., were used for each individual. In each experiment there were thirty-six fertilizations made, of which thirty were crosses, and six self-fertilizations. In the eight experiments that I carried out there were therefore 240 cross experiments, which, if not a very extensive series, yet ought to suffice to show the main points. The percentages given in the following tables indicate the proportionate number of eggs in each set that segmented; the estimated number are approximations only, and not, in all cases, the results of exact counts.

TABLE I.

A^{a}	A^{b} 90	A^{c} 80	A^{d} 10	A^{e} 0	A^{f} 0
B^{a} 0	**B**^{b}	B^{c} 3	B^{d} 0	B^{e} 0	B^{f} 0
C^{a} 100	C^{b} 80	**C**^{c}	C^{d} 100	C^{e} 75	C^{f} 0
D^{a} 90	D^{b} 75	D^{c} 80	**D**^{d}	D^{e} 0	D^{f} 0
E^{a} 20	E^{b} 25	E^{c} 0	E^{d} 0	**E**^{e}	E^{f} 0
F^{a} 0	F^{b} 0	F^{c} 0	F^{d} 0	F^{e} 0	**F**^{f}

An analysis of the results shown by this table leads to the following conclusions. The A-eggs were in good condition (or more briefly were "good"), since with "good" sperm 90 per cent. of them were fertilized in one case. The B-eggs were poor, the C-eggs excellent, the D-eggs very good, the E and F-eggs were poor. The a-sperm was excellent, as seen in C^{a}; the b, c, d, e-sperm were also excellent; but the f-sperm was very poor, since not a single egg in any of the sets was fertilized by it. From the results of this table it looks as though good sperm would always fertilize good eggs of any other individual, but there are even here three significant exceptions to this statement. The d-sperm gave poor results with A-eggs (A^{d}), although other experiments show that both the sperm and the eggs of these two individuals were good. The other cases are those of A^{e} and D^{e}, in which the e-sperm failed completely with the A and with the D-eggs, although with the C-eggs it gave 75 per cent. of segmenting eggs.

There was also another point that I wished to test by means of these experiments, namely, whether there is any correspondence between normal and abnormal development of the eggs and the proportion of the eggs that are fertilized. It will be recalled in this connection that Castle found that those cases of *Ciona intestinalis* in which self-fertilization occurs development is abnormal. On the contrary, I found that self-fertilized eggs often give rise to normal embryos. I wished, therefore, to see whether in cross-fertilized eggs there is any relation between the number fertilized and the condition of the embryos. In the following table the kinds of embryos that developed from the eggs given in the preceding table are recorded. The abnormal records generally indicate tadpoles that were bent, or crooked, or otherwise abnormal in shape.

TABLE I.—*Continued.*

A^{a}	A^{b} Abn	A^{c} Abn	A^{d} Abn	A^{e} 0	A^{f} 0
B^{a} 0	**B**^{b}	B^{c} Abn	B^{d} 0	B^{e} 0	B^{f} 0
C^{a} Abn	C^{b} Abn	**C**^{c}	C^{d} Abn	C^{e} Abn	C^{f}
D^{a} Abn	D^{b} Abn	D^{c} Abn	**D**^{d}	D^{e} 0	D^{f} 0
E^{a} Abn	E^{b} Abn	E^{c}	E^{d}	**E**^{e}	E^{f} 0
F^{a} 0	F^{b} 0	F^{c} 0	F^{d} 0	F^{e} 0	**F**^{f} 0

The table shows that all of the tadpoles were abnormal, despite the fact that a large percentage of the eggs was fertilized in several cases. The next table gives the results of a second experiment.

TABLE II.

A^{a} ·	A^{b} 10^{b}	A^{c} 40	A^{d} 50	A^{e} 0	A^{f} 40
B^{a} 30	**B**^{b}	B^{c} 90	B^{d} 80	B^{e} 1	B^{f} 25
C^{a} 90	C^{b} 95	**C**^{c}	C^{d} 99	C^{e} 100	C^{f} 100
D^{a} 90	D^{b} 90	D^{c} 80	**D**^{d}	D^{e} 1	D^{f} 99
E^{a} 0	E^{b} 40	E^{c} 25	E^{d} 50	**E**^{e}	E^{f} 0
F^{a} 1	F^{b} 40	F^{c} 80	F^{d} 15	F^{e} 0	**F**^{f}

The A and the E-eggs were only moderately good, the B, C, D, and F-eggs were very good. The sperm in all the sets gave very good results with at least some of the eggs. The exceptional cases found in this series were as follows. The a-sperm did poorly with B, very poorly with F, and gave nothing with E, although with C and with D 90 per cent. of the eggs were fertilized. The b-sperm also gave varying results, although the extremes were not so marked; and this holds to even a less degree with the c-sperm. The c-sperm gave exceptionally good

results with the F-eggs. There is nothing calling for comment in the behavior of the d-sperm; while the e-sperm was surprisingly deficient with the B and the D-eggs, although excellent with C. The f-sperm gave excellent results with the C and the D-eggs, but was less good with B. The later development of the eggs in this series was not observed.

TABLE III.

A^a	A^b 80	A^c 80	A^d 1	A^e 0	A^f 0
B^a 100	**B^b**	B^e 100	B^d 10	E^e 2	B^f 1
C^a 100	C^b 100	**C^e**	C^d 20	C^e 25	C^f 1
D^a 100	D^b 100	D^c 50	**D^d**	D^e 5	D^f 10 (1 in 10)
E^a 20	E^b 10	E^c 10	E^d 2	**E^e**	E^f 0
F^a 20	F^b 70	F^c 15	F^d 1	F^e 0	**F^f**

The A, B, C, D-eggs appear to have been excellent; the E-eggs poor, and the F-eggs probably good. The a and the b-sperm appear to have been good, and the latter, in the case of F, gave unexpectedly good results. The c-sperm was also good, but gave with D only 50 per cent. of segmenting eggs. The d, e, f- sperm was poor.

The embryos (tadpoles) were examined after 24 hours with the results recorded in the following table:

TABLE III.—*Continued.*

A^a	A^b Abn.	A^c Abn.	A^d Abn.	A^e One abn.	A^f 0
B^a Abn.	**B^b**	B^e Nearly norm.	B^d Abn.	B^e Abn.	B^f One norm.
C^a Norm.	C^b Norm.	**C^e**	C^d Most abn. One norm.	C^e Most norm.	C^f One norm.
D^a Most abn.	D^b Most abn.	D^c Abn.	**D^d**	D^e Abn.	D^f Abn.
E^a Abn.	E^b Abn.	E^c Abn.	E^d	**E^e**	E^f Abn.
F^a Abn.	F^b Abn.	F^c Abn.	F^d 0	F^e 0	**F^f**

Although A^b and A^e gave 80 per cent. of cases of fertilization they produced only abnormal tadpoles. B^a gave also only abnormal tadpoles, while B^e gave nearly normal tadpoles. Although only one egg in B^f was fertilized it produced a normal tadpole. The C-eggs gave even more curious results. The C^a and C^b-eggs gave exceptionally normal and active tadpoles; the C^d-eggs were abnormal (excepting for one individual); most of

the C^e were normal, while the one egg fertilized in C^f was normal. The D^a and D^b-eggs gave very abnormal tadpoles, but some of them were normal, while D^e and D^e and D^f were abnormal.

All of the E and the F-eggs gave abnormal tadpoles. These results show very clearly that there is no necessary relation between the percentage of eggs fertilized and the normal or abnormal development that takes place, although in general good eggs are more likely to produce normal tadpoles, and poor eggs abnormal ones.

TABLE IV.

A^a	A^b 15	A^c —	A^d —	A^e —	A^f —
B^a 30	**B^b**	B^c 35	B^d 5	B^e 0	B^f 0
C^a 5	C^b 0	**C^c** 1 in 10	C^d 0	C^e 0	C^f 0
D^a 20	D^b 10	D^c 3	**D^d**	D^e 8	D^f 0
E^a 100	E^b 100	E^c 95	E^d 100	**E^e**	E^f 0
F^a 75	F^b 1 in 10	F^c 4 in 26	F^d 1 in 50	F^e 0	**F^f**

In this series the A-eggs were not distributed through a mistake except in two cases. The B and the D-eggs were poor; the C-eggs very poor; the E-eggs were excellent; and the F-eggs seem to have been good. The a-sperm gave the best results, yet failed to give high percentages with the poorer eggs; and the b-sperm behaved similarly, but did less well with F. The c and the d-sperm were excellent with the e-eggs only, while the e and the f-sperm were poor in all cases, showing, in the case of the E-eggs, which were excellent, that the latter could not fertilize eggs as good as these.

TABLE V.

A^a	A^b 0	A^c 0	A^d 0	A^e 0	A^f 100
B^a 100	**B^b**	B^c 5	B^d 0	B^e 0	B^f 80
C^a 30	C^b 10	**C^c**	C^d 0	C^e 0	C^f 80
D^a 0	D^b 2	D^c 0	**D^d**	D^e 0	D^f 14
E^a 5	E^b 25	E^c 5	E^d 0	**E^e**	E^f 30
F^a 75	F^b 20	F^c 2	F^d 0	F^e 0	**F^f**

This experiment gave some curious results. The A-eggs must have been excellent to judge by A^f, yet gave no results in other cases. The B-eggs must have been excellent, but gave good results only with the a and the f-sperm. The C-eggs gave similar results, but few eggs segmented with the a-sperm. The D-eggs must have been poor giving only 14 per cent. with the f-sperm. The E-eggs also gave poor results,[1] and the F-eggs

[1] The E-eggs were polyspermic.

were well fertilized only with the a-sperm. The development of the eggs as shown by the following table gave some unexpected results when considered in the light of the number fertilized:

TABLE V.—*Continued.*

A^a	A^b Norm.	A^c Abn.	A^d 0	A^e 0	A^f Norm.
B^a Abn.	**B^b**	B^c Norm.	B^d 0	B^e 0	B^f Norm.
C^a Norm.	C^b Abn.	**C^c**	C^d 0	C^e 0	C^f Abn.
D^a ?	D^b Abn.	D^c 0	**D^d**	D^e 0	D^f Abn.
E^a Abn.	E^b Abn.	E^c Norm.	E^d 0	**E^e**	E^f Abn.
F^a Norm.	F^b Norm.	F^c 0	F^d 0	F^e 0	**F^f**

The B^a-tadpoles were abnormal although 100 per cent. of the eggs were fertilized; while the B^e-tadpoles were exceptionally normal. Although the c-sperm was very poor, the B^c and the E^e-tadpoles were exceptionally active and normal. The E^f-tadpoles were only somewhat abnormal, although the eggs appear to have been poor. The F^a and the F^b tadpoles were excellent, although only 20 per cent. of the eggs divided in the latter case.

TABLE VI.

A^a	A^b 0	A^c 0	A^d 20	A^e 0	A^f 0
B^a 100	**B^b**	B^c 25	B^d 75	B^e 65	B^f 95
C^a 90	C^b 90	**C^c**	C^d 90	C^e 90	C^f 50
D^a 0	D^b 2	D^c 0	**D^d**	D^e 20	D^f 20
E^a 20	E^b 95	E^c 40	E^d 100	**E^e**	E^f 80
F^a 1	F^b 95	F^c 30	F^d 100	F^e 40	**F^f**

The most striking results shown by this table are that while the F-eggs were excellent and the a-sperm also, yet F^a gave only one per cent. of segmenting eggs. The e and f-sperm gave much better results with D-eggs, than did any other sperm with these eggs, although some of the other sperm appears to have been excellent.

TABLE VII.

A^a	A^b 0	A^c 0	A^d 8	A^e 0	A^f 0
B^a 15	**B^b**	B^c 5	B^d 5	B^e 0	B^f 0
C^a 70	C^b 80	**C^c**	C^d 95	C^e 80	C^f 20
D^a 12	D^b 1	D^c 8	**D^d**	D^e 6	D^f 0
E^a 50	E^b 70	E^c 50	E^d 95	**E^e**	E^f 0
F^a 80	F^b 60	F^c 30	F^d 95	F^e 75	**F^f**

In this table also there are several cases where the sperm was less capable with some eggs than with others, even when other

cases showed that the eggs were equally good. The differences are not so striking as in some of the other series.

TABLE VIII.

A^a	A^b 20	A^c 85	A^d 75	A^e 0	A^f 35
B^a 0	**B^b**	B^c 20	B^d 25	B^e 0	B^f 0
C^a 0	C^b 0	**C^c**	C^d 8	C^e 0	C^f 0
D^a 8	D^b 50	D^c 90	**D^d**	D^e 0	D^f 7
E^a 0	E^b 8	E^c 80	E^d 50	**E^e**	E^f 0
F^a 0	F^b 3	F^c 8	F^d 15	F^e 0	**F^f**

With the exception of c- and of d-sperm the sperm appears to have been very poor in this series. The B, C and F-eggs were also poor. It is noticeable that the results appear to depend in this case more on the condition of the sperm than of the eggs.

In my previous paper five tables,[1] similar to these, were given for the *Ciona* found at Woods Hole. The incompatability was less marked, although some cases, apparently of this sort, were found. In general, however, the eggs crossed much more readily than did the California form. Since the individuals at Woods Hole were collected over a wider area than were those at Coronado Beach, all of which grew on the same float, the chance is greater that they may have had a different parentage. Whether the closer descent may account for the greater incompatability in the Coronado individuals can not be stated, but the facts are suggestive.

DISCUSSION OF EXPERIMENTS.

The most obvious question that suggests itself in those cases where few eggs were fertilized is whether if more sperm was added more eggs would segment. In answer to this I should point out that an approximately equivalent amount of the same solution containing sperm was added in each vertical series. Another experiment made to test this point showed that the amount added was many times greater than that necessary to fertilize the eggs when a good combination is made. Thus 3, 6,

[1] A few errors in my former tables may be corrected here. Thus in all the tables A^c appears twice, and E^a also. In experiment XIV. the first A^c gave 0 per cent., the second 100. This is due no doubt to the failure to add sperm to the first lot, which might easily occur. This is apparently also the case in experiment XV. In experiment XVI. the first E^a gave only 5 per cent., the last 70. This difference I cannot now account for. B^c in this table should be B^d, and C should be C^e.

12 and 24 drops of a solution containing the sperm was added to equivalent numbers of eggs, and gave 90, 90, 90, 100 per cent. of eggs fertilized. In another case 3, 6, 12 and 24 drops gave 6, 50, 90, 90 per cent. In the latter case three drops were not enough; six not quite enough; but 12 and 24 sufficed. Now since in all the other experiments more than 24 drops were used the outcome must depend in only a small degree on the quantity of sperm, and the results represent, therefore, approximately the proportion of eggs and sperm capable of uniting.

The tables show that the sperm is generally "at fault," when fertilization does not occur, the eggs being in nearly all cases capable of fertilization when good sperm is used, although, as pointed out, some striking exceptions occur. Thus poor sperm will sometimes fertilize more eggs of one set than of another, but the better the sperm the more eggs it will fertilize as a rule.

It is clear from the tables that it is erroneous to suppose that the sperm will fertilize equally the eggs of all other individuals. All degrees of sterility are met with in cases where other experiments with the same sperm fully succeed. Therefore the problem is not so sharply defined as appeared to be the case before these experiments were made.

Experiments to Determine the Nature of the Influences Preventing Self-Fertilization.

As stated above these experiments were undertaken in the hope of finding out whether there is a soluble substance in the eggs or body tissues of an individual that so affects its own sperm that it is rendered incapable of fertilizing its own eggs. If this is the case it would seem probable that the sperm might be affected by extracts of its "own" tissues, so that it might no longer be capable of cross-fertilization, if eggs of another individual were added to the extract containing the sperm.

The experiments to test this are not so simple as may appear on first thought, since check experiments must be carried out in order to see whether the same action may not result when extracts of the body of another individual are used. In fact it would have been very easy to make a serious blunder had not this precaution been taken.

The converse experiment should also be carried out at the same time; namely, that in which the sperm of A is put into the extract of B in order to see if it might not be made active so that it would then fertilize its own eggs. In my previous paper I have described some experiments of this kind. As a check to this the a-sperm should be put into the B-extract and then later the B-eggs added to show whether the extract itself may not interfere with the fertilization. The complete experiment is indicated in the following table:

A-sperm.	B-extract.	Add A-eggs	(1)
		Add B-eggs	(2)
	A-extract.	Add A-eggs	(3)
		Add B-eggs	(4)
B-sperm.	B-extract.	Add A-eggs	(5)
		Add B-eggs	(6)
	A-extract.	Add A-eggs	(7)
		Add B-eggs	(8)

Experiment 1. — In one experiment in which an extract of the ovary of the individuals was used none of the eggs segmented showing that even the extract of another individual prevents the cross-fertilization from taking place. A test experiment with these same eggs and sperm, brought together in sea water, showed that A-eggs with B-sperm gave only 20 per cent. of cases of segmentation, and B-eggs with A-sperm gave no cases of fertilization. It is clear, therefore, that the eggs and sperm were not in very good condition. When self-fertilized in sea-water neither the A-eggs or the B-eggs segmented.

Experiment 2. — In another experiment the eggs and the ovary of one individual (A) were soaked in sea water for one hour. The same was done with the eggs and the ovary of another individual (B). The sperm of A was added to the A-extract and the B-sperm to the B-extract for half an hour. Then the B-eggs were placed with the A-sperm that had been in the A-extract, and the A-eggs with the B-extract and B-sperm. None of the eggs divided. In this same experiment the B-sperm was put into the extract of the ovary of A, and later the A-eggs were added; similarly for the A-sperm. No eggs were cross-fertilized, showing as in the preceding case that the extract itself had interfered with cross-fertilization (provided the eggs were good which was not tested).

Experiment 3. — In this experiment the eggs (instead of the sperm) were soaked for a short time in the extract of the ovary of another individual in order to see if they might not acquire the power to become fertilized by the sperm if this were then added.

The A-eggs were soaked in the extract of the ovary of B about 15 minutes. The A-sperm was added to sea water, and left for 5, 10 and 15 minutes. Then the A-eggs were added to the A-sperm solutions. No eggs segmented, but neither did the eggs in the check experiment in sea water, showing that the eggs or the sperm were poor, or else incompatible. In the check experiment (B-eggs in extract of A-ovary), however, 100 per cent. of the B-eggs were fertilized by the A-sperm. This also happened in the cross in sea water. Here the A-extract did not prevent the B-eggs from being cross-fertilized by A-sperm. The solution must have been sufficiently dilute for fertilization to take place.

Experiment 4. — In this case the mantle was removed, and the body wall slightly cut so that the heart protruded, which was cut open and the blood collected. The eggs of another individual were then put into the blood. There is here a possible source of contamination, since some of the follicles of the testis may be broken, and allow some of the sperm to get into the blood. The results of one experiment of this sort were as follows :

			In Blood.	Diluted Later.
(1)	A-eggs	A-blood, B-sperm	0	40 %
(2)	B-eggs	A-blood, A-sperm	0	70
(3)	A-eggs	B-blood, B-sperm	0	0
(4)	B-eggs	B-blood, A-sperm	0	0

The check crosses carried out in sea water gave for the A-eggs (B-sperm) 90 per cent. of cases of segmentation, and for the B-eggs (A-sperm) 50 per cent. The first two combinations (1) and (2) of the table, gave no results in the blood itself, but when the blood was diluted they gave nearly the same percentages as in sea water. In the second cases the dilution was probably insufficient. Thus it is shown that the undiluted blood interferes with the fertilization, but on the other hand the diluted

blood of the same individual does not affect the sperm so that it fails to cross-fertilize.

Experiment 5. — The eggs were put into the blood of another individual for twenty minutes, and then removed to sea water, and their own sperm added. No self-fertilization followed, although direct cross-fertilization gave 70 per cent. for the B-eggs, and 100 per cent. for the A-eggs, showing that both eggs and and sperm were good.

Experiment 6. — In order to test whether the blood itself may not sometimes become contaminated with its own sperm, one portion of the blood of A was diluted with sea water, and B-eggs were then put into it. In one case a few eggs segmented, showing that contamination may occur. Heating the blood would, of course, kill the sperm, but this would open the experiment to the objection that the composition of the blood might be so affected that the postulated soluble substance is destroyed. Since most of the experiments gave negative results the contamination of the blood, if it occurred, would not vitiate such results; but if the results had been positive this possibility would havehad to be carefully reckoned with.

Effects of Shaking.

It seems not improbable that the covering of test-cells might be the immediate cause of the lack of power to self-fertilize. Therefore I tried the effect of shaking them off, and adding sperm of the same individual to the denuded eggs.

Experiment 7. — In this case only one egg out of 200 segmented, while 400 of the same eggs unshaken showed no segmentation.

Experiment 8. — In another case about 20 per cent. of the shaken eggs segmented, but some of those that segmented had still some of the follicle cells attached. In another instance 8 out of a total of 40, or 20 per cent., segmented.

These results show unmistakeably that shaking increases the percentage of self-fertilizations that take place, but whether on account of the removal of the follicle cells, or from some other change induced in the eggs is not certain.

Experiment 9. — In this case none of the shaken eggs segmented; although the crossed eggs gave 50 per cent. in one

case, and 100 in another showing that the eggs and the sperm were good.

Effect of Follicle Water.

If some substance in the follicle cells excites cross-fertilization, then it seemed likely that the water in which the eggs had been violently shaken, so that the follicle cells broke apart, might act on the sperm or eggs of another individual, and cause self-fertilization. This was tried in a few cases.

Experiment 10. — In one case five per cent. of the eggs divided; in three cases there were no divisions; in another case 70 per cent. divided, but went only into the two- or four-cell stages, which seemed to show that something unusual had taken place, and that the results were not due to the follicle water. There is, in fact, a source of contamination in this experiment that may fully account for all the cases observed. In removing the eggs from the oviduct some of the sperm from the vas deferens may be accidently squeezed out and become mixed with the eggs, and remaining in the follicle water fertilize the other eggs. The shaking might injure the sperm, but those that escaped might still suffice. The peculiar segmentation in the case in which a considerable number of eggs segmented may have been due, in fact, to the injury to the sperm. Separate experiments must be undertaken to test this possibility. The negative results in these cases are probably the more significant.

Effects of Standing.

Experiment 11. — The relative age of the eggs might, it seemed to me, be a factor in the result; also by standing in sea water the substance that prevents self-fertilization might be washed out. To test these possibilities, eggs were removed and kept for eight hours in one case in sea water and then self-fertilized with their own sperm that had remained in the animal. No results followed. That the eggs are not injured by standing in sea water was shown by their power to become cross-fertilized even after 24 hours.

Effects of Bursting the Eggs.

Experiment 12. — By compressing the eggs between a cover slip and a slide they can be burst in some cases. I attempted

then to self-fertilize the egg-fragments, etc. Many of the eggs that had been broken were self-fertilized, indicating that the resistance to self-fertilization is due to something in the membranes surrounding the eggs. The experiments need to be repeated on a larger scale, but even if placed beyond doubt, the explanation of what there is in the membranes that prevents self-fertilization remains still to be determined.

Extract of the Sperm.

Experiment 13. — Wishing to see if an extract of the sperm of one individual would cause the sperm of another individual to fertilize its own eggs, the following experiment was made. The sperm of three individuals was dried in the sun on a piece of glass; the residue, including the salt, was scratched off, and as much tap water was added as there had been sea water before. Sperm and eggs of another individual were then added to the solution, but no results followed.

Diluting the Water.

Experiment 14. — In order to determine whether dilution of the sea water might not bring about self-fertilization, the following experiment was made: One, two, three, four or five parts of tap water were added respectively to one hundred parts of sea water. In all of these solutions cross fertilizations took place, but in no case did self-fertilization occur.

Experiment 15. — In this case the eggs were first crossed and then put into sea water, diluted as follows: 40, 50, 60, 70 parts of tap water were added to 100 parts each of sea water. In the first and second solutions about half of the eggs segmented into several cells; in the third solution few eggs segmented, and none in the fourth, although they became polynuclear. The eggs of three individuals were put, with their own sperm, into the same solutions, but gave no results.

Summary.

1. It is not true that the sperm of a given individual will fertilize equally well the eggs of all other individuals.

2. The eggs appear to be in good condition much oftener than the sperm, but even good eggs cannot be fertilized *equally well* by good sperm of all other individuals.

3. In the case of poor sperm the discrimination shown between the eggs of different individuals is apparently more marked than in the case where the sperm is unusually good.

4. The results are not due to different amounts of sperm used in different cases, because the same, or nearly the same amount was used in each series. Other experiments showed that the amount of sperm used was many times greater than that sufficient to fertilize all the eggs present. On the other hand by using a larger amount of "poor" sperm the percentage of cases of cross-fertilization could probably be increased.

5. There is no definite relation between the number of eggs that are cross-fertilized and the normal or abnormal condition of the tadpoles. Previous experiments had also shown that perfectly normal tadpoles may be produced by self-fertilization in the few cases in which this takes place naturally or is induced.

6. If a strong extract of the ovary of an individual (A) is made, and the sperm of A (first made active in sea water) is added, and if then the eggs of B are also put in, they may not be fertilized if the solution is very strong, but if it is diluted fertilization may take place. Thus the A-sperm is not brought to rest by an A-extract, except in so far as the solution is too strong to allow any fertilization, as is shown when A-sperm is put into B-extract and B-eggs added — no fertilization taking place in the strong solution, but occurring if dilution is subsequently brought about.

7. If A-sperm is put into an extract of the ovary of B, and then A-eggs are added no fertilization occurs, showing that the extract of another individual does not excite the sperm to self-fertilization.

8. If the *eggs* of an individual (A) are placed in an extract of the ovary of B, then returned to sea-water and A-sperm added, self-fertilization does not occur. The extract of B does not effect the A-egg so that they will self-fertilize.

9. The blood and extracts of the body tissues of another individual give similar negative results.

10. Shaking the eggs, so that the follicle cells are removed, favors self-fertilization.

11. Placing the eggs and sperm of one individual in the "follicle-water" (obtained by shaking) of another individual gave distinctly increasing percentages of apparent self-fertilization, but the results are probably due to contamination.

12. Extracts of the sperm of other individuals did not, in the few cases tried, induce self-fertilization.

13. Dilution of the sea water does not facilitate self-fertilization, although cross-fertilization may still occur in water considerably diluted.

14. Eggs after standing for 24 hours in sea water are not fertilized by their own sperm.

15. Bursting the eggs, so that the membrane is ruptured or the egg is set free, allows self-fertilization to occur in a large number of cases, showing that the lack of self-fertilization of the unbroken egg is probably due to a resistance found in the surface of the egg, in the membrane, in the follicle cells or in their secretions.

Hypothetical and Speculative.

The immunity of the eggs of *Ciona* to its "own" sperm invites a comparison with cases of immunity to infectious diseases. The more so since Landsteiner and Metschnikoff have found that when, for example, spermatozoa of the ox are injected into the guinea-pig they remain active for some time, but if the injections of the ox sperm are continued at proper intervals the guinea-pig makes a spermatoxin that quickly brings to rest all later-injected spermatozoa. If something similar occurs in *Ciona* the reaction is much more delicate than any heretofore discovered, and would open a wide field for future investigation.

Without wishing at present to press this point too far it seems to me of sufficient weight to warrant calling attention to some of the implications in the case of *Ciona*.

The presence of eggs and sperm in the same individual might appear to give an opportunity for a reaction similar to that just described. If the eggs, and also the body tissue of the animal, were supposed to make a counter substance that would bring the spermatozoa to rest, we might account for the lack of self-fertilization in this way. Further, the proximity of the sperm-duct and the oviduct in *Ciona* suggests that the eggs might all the more easily be stimulated to form the substance.

There is also another possibility, viz., that a substance may be secreted by the sperm-duct that keeps the mature spermatozoa at rest, and that this substance may affect the near-lying eggs, so

that they also may acquire this property. Unless, however, this influence on the eggs were of a peculiarly individual sort, it would prevent all fertilization, cross- as well as self-fertilization, which is not the case.

There remains still another possibility which may better account for the conditions. It may be that the result does not depend on a reaction between the sperm and the tissues or eggs of the same individual, but to a similarity depending on the common descent of the sperm and the eggs. Owing to their close similarity of composition, the activity of the sperm on coming in contact with its "own" egg-membranes may be decreased, so that the spermatozoön can not force its way into the egg. This point of view offers certain advantages over the others mentioned, especially when extended to some other animals. Thus in the bee, the spermatozoa are stored up in the receptaculum of the female, but the fertilization of the eggs of the female is not thereby prevented. In this case the spermatozoa and the eggs have arisen from separate individuals, and, hence, fertilization is possible, despite the fact that the sperm is stored in the body of the female that contains the eggs. In some hermaphroditic animals and plants self-fertilization occurs, but this is not a fatal objection to the hypothesis, because, although in these cases also a condition similar to that in *Ciona* may be supposed to exist, it may not be sufficiently strong to prevent the sperm from entering the eggs —the activity of the sperm being greater, or the reaction being less marked. The fact that in some of these cases self-fertilization takes place less readily than cross-fertilization is distinctly in favor of the present point of view.

The greater activity induced in the sperm of *Ciona* by ether and other exciting substances may make them sufficiently active to break through the barrier around their "own" eggs, and having once entered the egg, the stimulus caused by the nucleus or by the centrosome may cause the development to proceed.

If the spermatozoa are brought to rest by substances of some sort on the surface of the egg, or in its membranes, these may be a part of the living substance, and not set free in the sea water. Hence the failure to detect such bodies in the water or extracts under the crude condition of the experiments.

Violently shaking the eggs may remove entirely, or in part, the protecting covering, so that self-fertilization may more often take place.

If eggs are shaken in sea water, so that pieces of the follicle cells are set free, these pieces would bring to rest all of their "own" sperm that came into contact with them, but the number of spermatozoa in the water might be too great for all of them to be caught, and those that remained might suffice to fertilize the eggs of another individual if they were then added to the water. Thus we can see why in the experiments cross-fertilization was not prevented when the sperm was first added to its own follicle water.

The fact that cross-fertilization was so often incomplete in the San Diego form, and so much more frequent in the form from Woods Hole, suggests that the difference may be due to the closer relationship of the former group of individuals. The Woods Hole individuals were collected over a wider area, and other conditions at this place make it more probable that these individuals may have had a separate descent. The close resemblance, in fact, between the infertility of *Ciona* when self-fertilized, and the infertility of closely inbred forms is apparent, and the same explanation may apply to both cases.

The immunity of the eggs of *Ciona* to sperm of the same individual suggests a comparison with the cases of immunity to infectious diseases, but it is evident that the two cases can not be identical, for the antitoxin of disease is supposed to combine with the toxin of the poison, and thus inhibit its action. It would be absurd to suppose that there are bodies in the egg large enough to lock up, as it were, the spermatozoa; but on the other hand it is not impossible that substances may exist that quiet the activity of the spermatozoa, and that these substances are a part of, or the immediate product of, the protoplasm of the individual, and are not produced by a reaction between the body tissues (or the eggs) and the spermatozoa of the same individual, as in the case mentioned above when the sperm of one animal is injected into another.

A THEORY OF THE NATURE OF PROTOPLASMIC RESPIRATION AND GROWTH.

A. P. MATHEWS.

Respiration is probably the most fundamental of living processes. It is common to all forms of protoplasm; as long as it persists, protoplasm is said to live; when it ceases protoplasm dies; and it is the point of attack of the most powerful poisons such as the isocyanides. Most of the other protoplasmic functions depend directly or indirectly on respiration as, for example, the discharge of impulses from the nerve cells, the beating of the heart and so on. The protoplasmic syntheses and many of the decompositions have been ascribed since Drechsel's work and that of Hoppe-Seyler to this fundamental process.

In any general theory of respiration the following facts have to be explained:

1. Even though surrounded by oxygen all protoplasm maintains itself while alive in a reduced state and acts as an intense reducing agent. Its reducing powers are comparable to those of nascent hydrogen.

2. For all forms of protoplasm free oxygen above a certain tension is an intense poison. For some forms this tension may be no more than a very small fraction of an atmosphere; for others it is from three to four atmospheres.

3. Atmospheric oxygen has little oxidizing power; whereas protoplasm brings about oxidation of the most radical nature and is comparable in its oxidizing powers to the most intense chemical oxidizing agents.

4. Hydrogen is evolved as a gas by a great variety of bacteria and moulds.

5. The production of carbon dioxide by protoplasm stands in no direct or immediate relation to the consumption of free oxygen.

6. Many forms of protoplasm such as the anærobic bacteria are able to bring about intense oxidations in the absence of atmospheric oxygen.

The only general theory of the nature of respiration which has been carefully worked out is that of Hoppe-Seyler.[1] According to this hypothesis there occurs in the cell a fermentative decomposition of some substance analogous in all respects to the fermentative decomposition of calcium formate by many bacteria.

$$(CHO_2)_2Ca + H_2O = CaCO_3 + CO_2 + 2H_2$$

By this fermentation nascent hydrogen is set free; the nascent hydrogen keeps the protoplasm in its reduced state; it combines with one atom of the oxygen of the air to form water and sets free the other atom as nascent oxygen which thus causes the intense oxidations of protoplasm. This hypothesis was attacked by Traube, who showed in many cases that the process included the formation of hydrogen peroxide which was the real oxidizing agent. Hoppe-Seyler demonstrated, however, that some of these oxidations were more powerful than hydrogen peroxide, which is not a very strong oxidizing agent, could produce.

With the discovery of the oxidases, Hoppe-Seyler's hypothesis has fallen into the background without the oxidase hypothesis giving a better explanation in its stead. The manner of action of the oxidases is still obscure; their chemical composition is unknown, and no oxidase will cause oxidations in the absence of oxygen, whereas many forms of protoplasm can carry out such oxidations in the presence of no free oxygen at all or only of traces of free oxygen.

The studies of Armstrong, Dixon and others on the processes of slow and explosive oxidations and combustions throw, in my opinion, a remarkably clear light on protoplasmic respiration. These authors have shown that in ordinary oxidation the presence of some water is necessary to the oxidation. Phosphorus in a perfectly dry state will not ignite in dry air. Armstrong[2] concludes that the primary oxidation in all these cases is not brought about by the gaseous oxygen, but by the water. The atmospheric oxygen acts the part only of a depolarizer to take care of the nascent hydrogen formed from the water.

[1] Hoppe-Seyler, *Physiologische Chemie*, I. Theil, Allgemeine Biologie, p. 126 ff. Berlin, 1877.

[2] Armstrong, *Chemical News*, July 15, 1904, vol. 90, p. 25; *Transactions Chemical Soc.*, vol. 63, p. 1088, 1903.

The formula for the rusting of iron on this hypothesis would be as follows :

$$\text{I.} \quad Fe + 2OH_2 = Fe(OH)_2 + 2H$$
$$\text{II.} \quad 4H + O_2 = 2H_2O \text{ or}$$
$$2H + O_2 = H_2O_2$$
$$H_2O_2 + Fe = FeO + H_2O$$

Reaction I. cannot go on unless oxygen is present to remove the nascent hydrogen. If no oxygen is present the nascent hydrogen at once reduces the iron oxide and forms metallic iron again. The atmospheric oxygen does not unite with the iron, but with the hydrogen.

The same hypothesis will explain protoplasmic respiration and at once makes clear the identity of anærobic and ærobic respiration. The following theory of respiration is founded in part on Armstrong's work.

The real respiration of all forms of protoplasm, both ærobic and anærobic, is brought about not by the oxygen of the air, but by that of the water. The hydrogen set free from the water combines with other elements of the protoplasm, thus keeping it reduced; it also combines with the oxygen of the air if this is present to form water; and in the absence of oxygen it may escape as free hydrogen.

The only difference between anærobic and ærobic respiration is that the anærobic protoplasm is so powerful a reducing agent that it is able to drive hydrogen out of the water, thus oxidizing itself without the aid of atmospheric oxygen to act as a depolarizer. Ærobic protoplasm being less powerfully reducing requires the presence of more or less oxygen to take care of the hydrogen. The difference between these different kinds of protoplasm is exactly the difference between metallic sodium and metallic iron. Sodium is so powerful a reducing agent that it oxidizes itself at the expense of water driving out hydrogen even in an atmosphere of hydrogen. This corresponds to anærobic protoplasm:

$$Na + HOH = NaOH + H$$

Iron is so weak a reducing agent that it requires the presence of free oxygen to take care of the hydrogen set free, before it will oxidize itself. This corresponds to ærobic respiration. Pro-

toplasmic respiration, therefore, is in reality not the consumption of gaseous oxygen and the liberation of carbon dioxide as ordinarily stated. We now know that the production of carbon dioxide stands in no direct causal relation to the consumption of oxygen. *Respiration is in fact the dissociation of water with the liberation of hydrogen.*

The evidences of the truth of this hypothesis are many and have been collected in part by Hoppe-Seyler. Hydrogen is produced and set free as such by a great many moulds and bacteria, *i. e.*, coli communis, penicillium, butyricus, etc. In the case of facultative anærobes like the colon bacillus, the hydrogen appears only if no atmospheric oxygen is present; it is burned to water if this be present. In many cases where the hydrogen is not set free as such it escapes as marsh gas or combines with the protoplasm or some of the constituents of the culture medium, such as levulose or sulphur. Thus as Hoppe-Seyler showed, in the fermentation of calcium acetate the hydrogen unites with the methyl or methylene set free by fermentation and comes off as marsh gas.

$$(CH_3CO_2)_2Ca + H_2O = CaCO_3 + CO_2 + 2CH_4$$

In the second place hydrogen is set free in the case of some bacteria which are able to oxidize substances in the absence of oxygen. In this case the oxygen can only have come from the water. Such a case has been reported recently by Mazé.[1] In the case of a certain bacterium alcohol was oxidized to acetic acid in the absence of air, if levulose was present. The levulose was at the same time converted into mannite. The reaction was probably as follows:

$$\text{I.} \quad C_2H_5OH + H_2O = C_2H_4O_2 + 4H$$
$$\text{II.} \quad 4H + 2C_6H_{12}O_6 = \underset{\text{Mannite.}}{2C_6H_{14}O_6}$$

Reaction I. can only go on in a positive direction or from left to right if some substance is present to remove the hydrogen and thus prevent equilibrium from being established.

It will be seen that as atmospheric oxygen acts the part only of a depolarizer any other oxidizing agent, that is any other sub-

[1] Mazé, *Annales de l' Institut Pasteur*, XVII.

stance which unites readily with nascent hydrogen can replace the atmospheric oxygen and permit oxidation to go on in the absence of air. In the example just cited levulose acts in this way. This principle is of considerable importance in bacteriology.

The hypothesis just stated necessitates, as will be seen, a total abandonment of the common interpretation of anærobic respiration as taking place at the expense of the oxygen of some food stuffs and shows at a glance the identity of the process in ærobic and anærobic forms.

What is the nature of the process going on in protoplasm by which this decomposition of water is produced?

A great number of reactions occur of just this type in organic and inorganic chemistry. One of the most striking is the oxidation of alcohol to aldehyde accompanied by the reduction of benzophenone to benzopinakon which occurs when an alcoholic solution of benzophenone is exposed to sunlight. The interpretation of this reaction as given by Nef's[1] brilliant hypothesis gives at the same time an interpretation of the living respiration which wonderfully simplifies the problem of the chemical basis of life.

According to Nef many organic reactions are brought about by a change in valence of the carbon atom from four to two. In the case just cited alcohol in the sunlight splits off water to some degree just as it does when heated and forms extremely active ethylidene particles as follows:

$$\text{I.}\quad CH_3-CH_2OH \longrightarrow \underset{\text{Ethylidene.}}{CH_3-CH=} + H_2O$$

If now benzophenone is present to act as a depolarizer the active ethylidene attacks the water and oxidizes itself to aldehyde setting free nascent hydrogen which reduces the benzophenone.

$$\text{II.}\quad CH_3-CH< + OH_2 \longrightarrow \underset{\text{Aldehyde.}}{CH_3CH=O} + 2H$$

$$\text{III.}\quad 2H + 2(C_6H_5)_2C-O = \underset{\text{Benzopinakone.}}{[(C_6H_5)_2CHO]_2}$$

It will be seen how exactly this process fulfills the requirements of the facts for a complete theory of respiration. Owing,

[1] Nef, *Liebig's Annalen*, vol. 335, 1904, p. 192.

therefore, to Nef's hypothesis, and without this the exact mechanism of the process would be obscure, the following picture of protoplasmic respiration may be formed:

In protoplasm there is some substance (or substances) of unknown nature which splits off water from itself just as the alcohol does in the above reaction or as ammonium hydrate does and sets free from itself active particles having the properties of ethylidene. The following three well known reactions are exactly similar to this primary process:

$$NH_4OH \rightleftharpoons H_2N< + H_2O$$
$$H_2SO_3 \rightleftharpoons O_2S< + H_2O$$
$$C_2H_5OH \rightleftharpoons CH_3-CH< + H_2O$$

For protoplasm the reaction is as follows:

$$R-CH_2OH \rightleftharpoons R-CH< + H_2O$$

In this reaction R—CH_2OH is some unknown substance.

The nature of the substances thus reacting in protoplasm is unknown. There is no evidence, however, nor is there any reason for assuming that they are complex substances. It is probably not a substance acting by means of a change in valence of nitrogen for the reason that the nitrogen substances such as ammonia do not have so great an affinity for oxygen as that required of the unknown substance. We do not get for example such a decomposition of ammonia as this:

$$NH_3< + OH_2 = H_3NO + H_2.$$

On the other hand the substance possesses the powers ascribed by Nef to bivalent carbon. Carbon has a powerful affinity for oxygen. It may well be, therefore, that the substance is a carbon compound, presumably of simple nature and derived directly or indirectly from the foods so that new raw material for the reaction is constantly supplied. If this is the case Nef's view that bivalent carbon is at the bottom of the vital reaction would be justified.[1]

[1] Probably all reducing agents act in some measure in this way. For example in egg white a substance is present which forms sulphuretted hydrogen in the presence of sulphur. This substance is of unknown nature. The reaction probably is as follows:

$$X-CH< + OH_2 = XCHO + 2H$$
$$2H + S = H_2S$$

Whatever their composition may be the active particles attack the water of the protoplasm, oxidize themselves to aldehydes and set free nascent hydrogen.

$$R-CH< + OH_2 = R-CHO + 2H.$$

This explains the presence of aldehydes in protoplasm; the production of hydrogen by many or all forms of protoplasm as assumed by Hoppe-Seyler and the keeping of the cell protoplasm in a reduced state. It is perfectly clear that surrounded by oxygen as most protoplasm is it could not possibly continue in its reduced condition if some strong reducing agent such as hydrogen was not constantly produced. If too much free oxygen is present the aldehydes will be converted into acids, the reaction of the protoplasm changed and the life of the cell destroyed. Oxygen in other words above a certain pressure is poisonous for protoplasm.

In those forms of protoplasm called anærobic the active particles are of such a character and so powerful that in the absence of oxygen they can like sodium, oxidize themselves and set free hydrogen; or in the presence of certain foods which will combine with hydrogen such as levulose, they are able to oxidize themselves. In those forms of protoplasm called ærobic the active particles are not so powerful and require the aid of atmospheric oxygen to combine with the hydrogen before they can decompose water. While the active particles are formed spontaneously, the conditions in protoplasm may be such as to accelerate their formation (ferment action, see Nef).

A few examples will perhaps make clear the possible relations of this fundamental reaction to the synthetic properties of the protoplasm. The fundamental nature of most of these syntheses is simple and consists in the elimination of water between two simple molecules to form a complex molecule, as for example, in the condensation of the amino acids to form albumin; of the monosaccharids to form disaccharids and polysaccharids; of the amino acids to form pyrimidin; of the alcohols and fatty acids to form fats; of ammonia and oxyacids to form amino acids.

Two explanations of this process may be given. That of Drechsel is the first. According to this theory the elements to

form water are eliminated by a successive oxidation and reduction of the substances. A type of this reaction is the conversion of ammonium carbamate into urea.

$$NH_4OCONH_2 + O = NH_2OCONH_2 + H_2O$$
$$NH_2OCONH_2 + 2H = NH_2CONH_2 + H_2O.$$
Urea.

Upon the basis of this hypothesis, Drechsel succeeded outside the cell by rapidly alternating reductions and oxidations produced by induction shocks in obtaining urea from albumin; in synthesizing hippuric acid from benzoic acid and glycocoll, and in synthesizing ethereal sulphates. These syntheses may thus very readily be the secondary result of that fundamental respiratory process characterized as it is by a simultaneous reduction and oxidation.

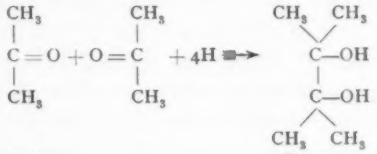
The syntheses may also be due as shown by Nef for many reactions to the condensation of active particles, that is particles formed by the dissociation of organic substances and containing open valencies. A typical synthesis of this sort would be the formation of olefine gas from methyl alcohol, or the condensation of the isocyanides.

I. $2H \cdot NC \rightarrow HNC< + HNC< \rightarrow H_2N_2C_2$

II. $2CH_3OH \rightarrow 2CH_2< + 2H_2O \rightarrow C_2H_4 + 2H_2O$
Olefine.

If in protoplasm conditions are such as to cause a good many active particles to be produced, syntheses by condensations of all kinds must spontaneously and of necessity occur. In this way it is seen that both growth and respiration are due to the spontaneous formation of active particles from the foods.

In the third place, mere reduction may cause many syntheses. A typical example of this sort is the condensation of acetone by nascent hydrogen.

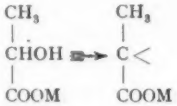


Acetone. Pinacone.

In this case a forked chain is produced, in which the carbon atoms are attached directly.

Moreover, from the aldehydes produced in the way indicated, all manner of compounds will originate spontaneously, as Loew has particularly pointed out. Thus from formaldehyde, formose and the sugars spontaneously originate in faintly alkaline solutions; glycerine aldehyde goes over of itself into a hexose sugar; acetic aldehyde in the presence of ammonium cyanide forms propionic amido-nitril, which readily passes into amido propionic acid or alanin, one of the most common constituents of the albumins. The albumins themselves are nothing else, as Kossel and Fischer have shown, than condensed amino acids. This condensation, there is every reason to believe, can be brought about by cell extracts, since from the kidney a substance has been isolated which brings about identically the same kind of a condensation, namely, that of benzoic acid and glycocoll, to form hippuric acid. Finally, by spontaneous union of the aldehydes with the cyanides found in so many cells, carbon chains are built up readily outside the cell and presumably in the cell also, since nitrils are not uncommon constituents of protoplasm.

Many other examples might be given here, but these will, I think, indicate how completely the formation of substances found in protoplasm can be accounted for by means of this fundamental reaction which is going on. These syntheses and decompositions must take place as a matter of course if the reaction is of the nature sketched. I do not mean to imply that the amino acids, for example originate altogether in the manner indicated. They may also be formed by the union of oxyacids dissociated in Nef's sense with NH_3, without the intermediate formation of aldehydes or ketones. Nef has shown that the lactates for example dissociate as follows :



Lactate.

If now NH_3 is present, union takes place directly with the bivalent carbon atom.

$$\begin{array}{l} CH_3 \\ | \\ C< \\ | \\ COOM \end{array} + \begin{array}{l} H \\ NH_2 \end{array} = \begin{array}{l} CH_3 \\ | \\ HC-NH_2 \\ | \\ COOM \end{array}$$

Alanin.

In any case it will be apparent that the synthetic powers of the cell depend on the same process as the respiratory powers.

While the interpretation of the exact mechanism by which the respiratory substance or substances (for there may be many such substances in one cell) dissociate water depends, as will be seen, upon Nef's hypothesis and is due to this hypothesis, the general hypothesis that such dissociation of water is taking place and that this is the basis of respiration, is independent of Nef's hypothesis. If, however, that hypothesis be accepted, and of its truth he has already produced so many proofs as to entitle it to provisional acceptance, we can go farther and ascribe this most fundamental of living reactions and many protoplasmic syntheses to methylene dissociation, that is, to bivalent carbon particles in protoplasm, as Nef himself has pointed out. Protoplasmic respiration must in any case be defined in the light of known facts somewhat differently from the ordinary statement.

Respiration is that process going on in protoplasm by which water is decomposed into oxygen and hydrogen. The oxygen combines with the substances of the protoplasm thus oxidizing them; the hydrogen is either set free in the gaseous form, or it is united with atmospheric oxygen to form water; or it combines with other substances in the protoplasm.

The reaction may be written as follows:

For anærobic protoplasm:

$$\text{I.} \quad R-CH_2OH \rightarrow R-CH< + H_2O$$

$$\text{II.} \quad R-CH< + OH_2 \rightarrow R-CH=O + H_2 \text{[1]}$$

For ærobic protoplasm:

$$\text{I.} \quad R'-CH_2OH \rightarrow R'-CH< + H_2O$$

$$\text{II.} \quad R'-CH< + OH_2 + O \rightarrow R'CH=O + H_2O, \text{ or}$$

$$\text{III.} \quad R'-CH< + OH_2 + O_2 \rightarrow R'CH=O + H_2O_2$$

$$\text{IV.} \quad R'-CH< + H_2O_2 \rightarrow R'-CH=O + H_2O$$

[1] A great number of reactions of this type are given in Nef's paper already referred to. See also Nef, *Journal of the American Chemical Society*, Vol. XXVI., 1904, p. 1549.

The views expressed in this paper may be harmonized with those in a previous paper in which the oxidations and reductions going on in protoplasm were regarded as electrical,[1] if the following explanation of the dissociation of NH_4OH into NH_3 and H_2O be adopted. At present no clear connection between ionic dissociation and such dissociations as that of ammonia has been established. The relation of the two processes may be the following, if Thompson's electron hypothesis be adopted.

In sodium chloride the sodium and chlorine are held together by the affinity each has for a negative electron. Na—**θ**—Cl. On going into aqueous solution the atoms separate and chlorine having the greater affinity for the electron (+ 1.965 volts to − 2.54 volts for the sodium in normal solution) takes it away from the sodium. The sodium being thus left with an unsaturated affinity for a negative charge becomes a positive ion; the chlorine with a free negative charge becomes a negative ion. The free charges being on separate atoms and free to move the solution conducts the current. In the albumin molecule of amphoter reaction hydrogen ions are dissociated at one place; hydroxyl at another. A free positive charge and a free negative charge reside on the same molecule though on different atoms. The albumin molecule may, therefore, be unable to take part in the conduction of the current although ionized.

$$H\overset{+}{\text{—}}\quad \theta\text{—albumin—}\overset{+}{\quad}\quad \theta\text{—OH}$$

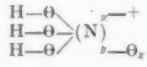
In the nitrogen atom as it exists in ammonia the opposite charges are on the same atom. The condition may be represented as follows where nitrogen is trivalent.

$$\begin{matrix} H\text{—}\theta \\ H\text{—}\theta \\ H\text{—}\theta \end{matrix} \Big\rangle \overset{x}{(N)}_{y} > \theta_z$$

If N is the nitrogen atom it has three valencies satisfied by hydrogen. At x and y, two points on its surface, there is a positive charge not entirely compensated by the atom itself. This charge is compensated by the electron z. This electron may be regarded as holding together the two parts of the nitrogen atom at x and y, just as the electron held together the separate atoms

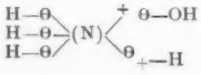
[1] Mathews, *Amer. Journal of Physiology*, 1904, X., p. 290.

of sodium and chlorine. In this case, I assume, the condition is that of Nef's polarized valencies. Particles in this condition he assumes to be inert so far as valencies x and y are concerned. Nef assumes that these polarized valencies open up and they open up more readily under some conditions than under others. For example, the polarized valencies of the carbon atom open up when the carbon is joined to nitrogen as in the isocyanides far more easily than they do when the carbon is joined to oxygen, as in carbon monoxide. The opening up of the valence is, I think, identical in character with the dissociation of a salt into its ions. The opening of these valencies constitutes a dissociation of valencies. It makes, of course, no difference theoretically whether those valencies are on the same atom or on different atoms. At any rate NH_3 goes over into:



These are Nef's active particles. It is only when in this condition of dissociation that these valencies will combine. One of these valencies (x) is positive; the other (y) is negative. Such particles, although they are really in an ionic form, cannot conduct the current because they are positive and negative at the same time.

These particles combine as follows:



Exactly the same reasoning applies to all atoms, including bivalent carbon. It will be seen, therefore, that this explanation which is but a trifling addition to the fundamental idea of Nef of polarized valencies, would bring the respiratory reactions into the domain of electrical reactions. No doubt this explanation has occurred to others, and I give it only as a convenient picture. It bridges so well the gap between Nef's views of organic reactions and Ostwald's hypothesis that inorganic reactions are ionic that it may be useful.

It will be noticed also that upon this view residual valencies may be pictured as polarized valencies and in each case both pos-

itive and negative valencies are opened simultaneously. The number of residual valencies may be considerable since upon Thompson's hypothesis of the electronic constitution of matter the movement outward of any electron owing to the speed of rotation of the atom or a disturbance of its electrical equilibrium would open up such valencies. In fact, the whole atom would be made up of polarized valencies if we look at it in this way.

Summary and Conclusion.

The foregoing hypothesis has a close bearing on the spontaneous origin of living matter. As a result of chemical and histological work it is now clear that living matter is a mixture of various substances and is not a chemical compound; there is no living compound. While this conclusion may not be admitted by all, it is, in my opinion, clearly established. There is no longer any doubt that the different phenomena of life are due to different constituents in this mixture. The histological, physiological, pharmacological and chemical evidence is unanimous on this point. The problem of the origin of life no longer appears, therefore, under its former guise, of how the foods are transformed into a living molecule or compound, but in its place we have instead, how are the foods transformed into the mixture of substances which is called living matter.

The question which we have to answer is this: is the transformation of the foods into living matter produced by the foods; or is it produced by the living matter? At first glance everyone would say that the transformation was caused by the living matter. This is the answer everyone has given hitherto and its truth appears to be self-evident. If we mix the foods by themselves they do not produce living matter. This answer has made it difficult to see how living matter originated on the earth. Preëxisting living matter was always needed to get living matter.

In the light of the foregoing discussion and the study of the transformations of the foods in protoplasm it appears highly probable that this answer is erroneous. Living matter does not cause the transformation of foods into living matter as we imagine; instead the foods to-day spontaneously change themselves into living matter just as they did at the beginning. The change goes on very rapidly in living matter, but very slowly outside.

The cause of the formation of living matter is to be sought in the foods and not in the living matter.

As this conclusion has not so far as I know been hitherto perceived, the reasons for it may be briefly presented. They are in brief two: The examination of protoplasm has shown that it contains a great number of catalyzing agents or ferments. So many of these have been found in all cells and so clearly do protoplasmic reactions partake of this nature that the opinion is widely accepted that the chemical transformation of the foods in protoplasm into the substances constituting protoplasm is brought about by ferments. The work which has been done by the physical chemists and others upon ferments shows in the clearest manner that ferments are substances which do not cause reactions, they only accelerate reactions which will go on anyway in their absence, but which go on very slowly. Ferments therefore are accelerators of spontaneous reactions. Zymase does not cause the decomposition of the sugar molecule, it only accelerates its decomposition. The necessary result of this fundamental conception of the nature of ferment actions is this: the nature of the chemical transformations which the foods undergo in protoplasm is not altered or determined by the protoplasm. The nature of the transformation is determined by the foods; protoplasm by means of the ferments it contains only influences the *rate* of the transformation. This means as already pointed out that the foods must spontaneously transform themselves into the mixture called protoplasm, if given time enough. In protoplasm this transformation goes on very rapidly because some of the products of the reaction act as catalyzers to hasten the rate of this or that phase of the reaction.

In the second place a careful study of the transformations of the foods within and outside of protoplasm has failed to show a single instance in which the character of the transformation is different in two cases. This result is now so well understood in physiological chemistry that if we wish to discover what substances are formed out of any food or other substance during its passage through protoplasm, we subject the substance to decompositions, hydrolytic, oxidative, or reducing, outside the body, determine the substances formed and then look for these substances in the organism knowing that they will be formed there also.

In the third place a mass of evidence shows that substances outside the body simply dissolved in water, break up at a very slow rate, but into the same compounds in many instances as appear at a rapid rate in protoplasm.

These facts may be summarized in the general statement: Living matter does not determine the *character* of the transformation of the foods; it only determines the *rate* of transformation.

This conclusion at once makes clear that living matter is to-day originating spontaneously from the foods just as it always has. The transformation goes on now very rapidly owing to catalyzers in protoplasm, whereas originally the transformation probably went on slowly until some of the products of the reac tion were produced which acted as catalyzers for this or that phase. What we call living matter is of importance in the process only because it contains from the outset these end-product catalyzers, formed from the previous reaction. The whole process is, I conceive, as follows:

The carbon constitutents of the foods spontaneously decompose. By this decomposition particles in a nascent state are formed. Upon Nef's hypothesis these would be particles with bivalent carbon. Whatever their nature these nascent particles either act on the water, oxidizing themselves to aldehydes or ketones and setting free hydrogen and in this manner causing protoplasmic respiration; or they combine with each other to form the various constituents of protoplasm and thus cause growth. What they combine with depends on what substance is near when they become nascent. If it is ammonia amids, amino-acids and other nitrogen compounds are formed; if another carbon compound, the carbon chains are built up and the complex substances which make up protoplasm. The whole process is due to a spontaneous dissociation of the food molecules and is not due to any vital energy. Some of the end-products of this spontaneous rearrangement act as ferments, that is, as accelerators of some phase of the reaction, or they may act as negative catalyzers delaying some phase. Examples of such processes are well known in chemical reactions. In exactly what manner they accelerate or delay the reaction is not certain.

The bearings of this conclusion upon many problems of biology, and particularly upon the problem of differentiation in

development is obvious. The substances produced from the foods depend on the foods and not on the protoplasm. The so-called organ-forming substances of the egg described by Whitman, Lillie, Conklin and others, determine necessarily the character of the protoplasm formed from them. There is but one other factor to be considered, *i. e.*, the presence of catalyzers which may accelerate different phases of a reaction. By this means a food substance, undergoing in many different cells the same course of transformation, will give rise in each to different proportions of substances, depending upon what stage of its transformation is accelerated. Thus, for example, a sugar undergoing one transformation into alcohol, lactic acid and carbon dioxide, or other substances may very readily give rise almost exclusively to lactic acid, if the proper phase is accelerated.

In this way, it is possible to see how the same foods in different protoplasms will form substances which are present in the different cells in widely different proportions. A specific instance will make this clear. Amino-acids spontaneously split off the amid group and form oxyacids. This occurs at a very slow rate and is a reversible change. In the liver of mammals there is a catalyzer which greatly accelerates this transformation. The result is that in this organ quantities of ammonia are produced and a non-nitrogenous residue. In cells lacking this ferment, this reaction goes on so slowly that there is no opportunity for the accumulation and farther decomposition and recombination of the products thus set free. Numerous other examples will occur to all physiological chemists.

In conclusion, I wish to point out that this conception is in many important particulars only an application to some of the problems of physiological chemistry of Nef's theory of the nature of organic chemical reactions. As will be seen from this paper, I believe that his hypothesis of the spontaneous decomposition of organic molecules with the formation of extremely reactive dissociation products throws a new light on the chemical transformations in protoplasm, and that many of those reactions become clear at once if it be assumed that the reactive particles have the properties of bivalent carbon. The importance of this theory for physiological chemistry is not yet sufficiently recognized.

ON THE NUCLEOLI IN THE SOMATIC AND GERM CELLS OF PEDICELLINA AMERICANA.

LOUIS I. DUBLIN.

In the course of a work on the germ cells of *Pedicellina americana*, just published, (Dublin, '05), my attention was called among other matters to the study of the character of the nucleolus, not only in the various stages in the differentiation of the germ cells, but in the somatic cells as well. Thus, in the resting condition of the cell, the nucleoli are nearly everywhere in pairs and are situated at opposite points near the nuclear membrane. This arrangement is so constant, as are also the later processes through which these bodies pass, that the possibility was immediately suggested that *Pedicellina* might be one more instance among the many cited by Häcker, '02, where there is a distinct outward expression of the internal independence of the paternal and maternal elements, not only in the nuclei of the early somatic cells, as is most clearly observed in several forms, but also in the early germ-cells. It was therefore determined to put this whole question to a test by an examination of the cells throughout the whole life cycle, *viz.*, to observe whether the condition of the chromatin was actually in accordance with the conditions so strongly suggested by the appearance and behavior of the nucleoli.

For this purpose *Pedicellina* presents very favorable conditions. In the first place, owing to the comparatively small size of the polyps, the various tissues including the ovaries and testes, can be brought into the same microscopic view, thus affording very close comparison of these structures throughout. In the second place, and more important, owing to the nature of the budding process and the internal development of the embryo, the cells of all the stages of the life history are easily accessible for study with the exception of a short and, for our purpose, rather unimportant period during metamorphosis when the free swimming larva leaves the brood-pouch and becomes attached. Such a study of the entire life history is, finally, all the more important

in the light of the discussion on the nature of the nucleoli and of the relation which the latter bear to the chromatic substance. I shall then, first, review the character of the nucleoli throughout the life history and, second, limit myself to the nucleolus of the growing oöcyte.

I.

The youngest polypides at my disposal were those which had but lately passed through their metamorphosis (Fig. 1). These in many cases do not as yet show any traces of the budding stolon and have certainly no genetic connection with the colonies among which they are found. There is still no apparent distinction of sex and the internal structures are at the beginning of their development. Of the digestive system, only the middle portion has definitely formed. The atrium or vestibule is represented by a small space which has as yet no connection with the outside. Surrounding the gut and vestibule and filling the space between these parts and the body wall are masses of embryonic cells which later give rise to the ovary or testis to the nervous and excretory systems, the œsophagus and rectum, the tentacles and the few muscle bands. These embryonic cells are all of one main type and cannot as yet be distinguished very positively from the primitive germ-cells which lie in their midst. One character, however, stands out strikingly. The comparatively large nuclei, in the great majority of cases, contain each two nucleoli. These two bodies are located symmetrically with respect to the halves of the nucleus. In the remaining cases there is but one nucleolus and this one is almost invariably of larger size than either of the two and is located at or very near the center of the nucleus.

FIG. 1.

In a somewhat later stage, the stalk is longer and the internal organs begin to show something of their permanent form. The primitive germ-cells, which can now be distinguished very clearly, have taken their final place between the "liver-cells" and the

floor of the atrium and there multiply very rapidly. This increase is much more pronounced in the male than in the female and gives the first and principal basis for the distinction now present between the sexes. The bi-partite testis, with its spermatogonia, has grown considerably and the latter are mostly in the resting condition. The nuclei are large, being surrounded by a very thin layer of cytoplasm. Within the nucleus, the chromatin, in the form of a reticulum, stains very lightly; indeed, in well extracted iron-hæmatoxylin preparations shows scarcely at all. But what is more important is the almost invariable presence of the one or the two nucleoli. These stain uniformly and intensely black with hæmotoxylin and retain their color long after that of the other cell elements has been extracted. When in pairs, they are placed at opposite points of the nucleus; when single, the nucleolus is found more nearly in the middle. The cells of the other tissues have preserved the same condition of the nuclei as was above described for the younger polyp.

In older testes, many more cells are present (Fig. 2), and these are proved, by the unreduced number of the chromosomes, to be also spermatogonia. Among these are cells of many sizes both

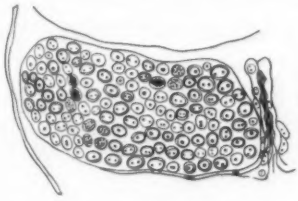


FIG. 2.

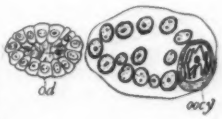


FIG. 3.

at rest and in mitosis from which it is certain that there are several generations of spermatogonia. In tracing the development of the spermatogonium from the telophase of the preceding division, attention is attracted by the appearance of the two nucleoli. At the beginning of the reconstruction there is as yet no trace of them. This stage is soon followed by one where the nuclear membrane has reappeared, the chromatin being almost entirely unaffected by hæmatoxylin. At two opposite points of the nucleus there now appear the two nucleoli as yet very small but

deeply staining, very much like the granule of a centrosome. It is important to observe, that the number is at this point always two, the one larger nucleolus occurring only in the later stages of spermatogonial development. The chromatin soon increases in staining power, the nuclear membrane stands out more clearly and the two nucleoli increase in size until they become very conspicious. In the Auerbach preparations, the green chromatin reticulum near the nuclear membrane stands out rather clearly from the two red plastin nucleoli. These, at this point, approach the center of the nucleus and finally fuse into one larger nucleolus. All the stages in the approach of the two are to be found in one microscopic field in almost any testis. The appearance of these bodies as they come into closer relation, indicates that there is a flowing of their liquid substance toward a common center. In this way is produced a fine strand, deeply staining, which connects them before they have fused. We can now more readily understand the prevalence of the single nucleoli in resting spermatogonia. They represent the two, which, originally distinct, have now fused. It must here be added, however, that there is some doubt whether the two always fuse into one. There is evidence for the possibility that, in some few cases at least, the two remain distinct until the formation of the next spindle figure when they disintegrate.

The chromatin by this time stains much more intensely and takes the form of definite chromosomes. These may now move from the periphery and come into closer relation with the one or the two nucleoli. This connection is, however, always a secondary one, and in no case represents the origin of chromosomes from chromatin nucleoli. There can be little doubt of this conclusion, for on staining with Auerbach's fluid the chromatin stains intensely green while the nucleoli are always red. The same decisive results were obtained with the use of the Borel stain.

The oögonia at first resemble the spermatogonia very closely (Fig. 3) but can be distinguished, even at an early period, by their smaller number. The chromatin goes through exactly the same transformation in the several generations as do also the two nucleoli. In their early appearance at or near opposite points on the newly formed nuclear membrane, in their subse-

quent growth and movement toward the center, and finally, in their fusion, the resemblance is quite complete. We may therefore infer that whatever be the significance of the regularity of the occurrence of these bodies in the males must also hold in the case of the females.

This striking condition of the nucleoli of the primary germ-cells (Ur-genital Zellen) and of the several generations of spermatogonia and oögonia can be no mere chance occurrence. In the testes of older individuals, where some of the spermatocytes have already arisen, nearly every resting spermatogonial cell shows the primarily double or the secondarily fused condition of the nucleoli. This period closes, however, with the spermatogonic cycle since the spermatocytic divisions follow without an intervening resting stage. In the ovary, where the last oögonial division is followed by a long period of growth, the young oöcytes also show the same condition of the nucleoli that has been described for the oögonia. These either persist separately or fuse, but, unlike the nucleoli of the earlier germ-cells, become the composite structures generally found in the nearly matured egg. To this, however, we shall turn below.

As was pointed out in the discussion of the young polypides, where the internal tissues are not as yet completely differentiated,

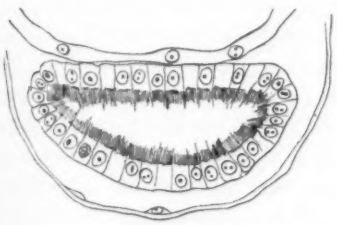


FIG. 4.

FIG. 5.

the primary germ-cells are not to be distinguished either in size or form from the other embryonic cells. These latter also show the same double nucleoli and act in other respects as do the germ-cells. From this we might expect to find similar conditions in the several tissues of the body to which the embryonic cells give rise, and such is, in fact, the case. In polyps, old or young, in males or in females, every organ shows the same con-

stitution of the nuclei. In the highly-modified "liver-cells," in the epithelium of the gut (Fig. 4), in the cells of the atrium and tentacles (Fig. 5), the primitive conditions are alike preserved.

From the universality of the occurrence of this phenomenon, it is difficult to escape the conviction that we are here concerned with conditions of considerable importance in the ultimate constitution of the nucleus. We must now inquire what light the fertilization and the early cleavage stages will throw on the problem; for it is here that the constitution of the nucleus ought most readily to be made out. In the first place, the two pronuclei do not completely fuse in the act of fertilization. In this regard, *Pedicellina* recalls the condition found by many observers in other forms. Indeed, the individual chromosomes are often formed in both pronuclei before their apposition has occurred and in no case is there much, if any possibility of their fusion during

FIG. 6.

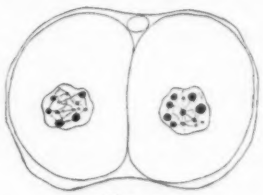


FIG. 7.

fertilization. The nuclei are extremely large and apparently correlated with this is a fact that each contains many nucleoli; the number varying from two to as many as seven or more (Fig. 6). In the 2- (Fig. 7), 4-, and 8-cell stages, the same conditions with reference to the nucleoli exist. As the cleavages go on a little further the conditions become more in accordance with what was observed in the latter somatic stages. The nuclei become successively reduced in number and it is no uncommon occurrence to find in a 16-cell stage as many as half the cells with two nucleoli (Fig. 8). These two may fuse into one, as is the case in the oögonia and spermatogonia already described. In the 32–64-cell stage, the mono- or bi-nucleolate condition is already the all-prevailing one, all the stages in the approximation and fusion of the two into one being present (Fig. 9). From this point forward, I have never found, in any of the many em-

bryos examined, more than two nucleoli and these go through all the typical transformations already described. The same conditions are obtained in all the resting cells of the later embryos up to and including the free-swimming larva (Fig. 10). After

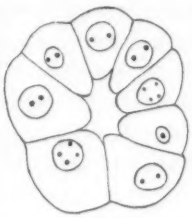


FIG. 8.

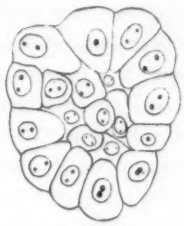


FIG. 9.

the fixation of the latter, and the ensuing metamorphosis, the young polypide, before stolon formation, shows the same interesting conditions. We have thus traced the constitution of the nuclei from the early polyp through the maturer ones, into the formation of the germ-cells and finally, in the new generation, have passed through the cleavage stages up to the formation of the larva and have completed the cycle with the fixed polyp once more.

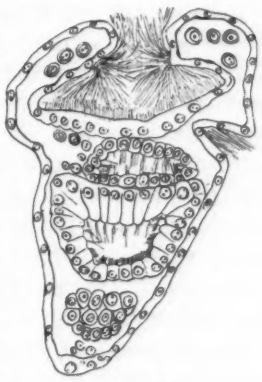


FIG. 10.

From this evidence, coupled with that of Rückert, '95, Häcker, '95–'02, Zoja, '95, Herla, '93, Conklin, '01–'02, and most recently of Moenkhaus, '04, one might at first thought conclude that in *Pedicellina* as in the Copepods, etc., the nucleoli actually express the internal relations of the parental chromatins of the nucleus and in their duality represent the persistent segregation and autonomy of the paternal and maternal chromosomes. This conclusion, however attractive, has on close examination of the evidence not only in *Pedicellina*, but in the other forms as well, a source of serious difficulty. From the recent work of Moenkhaus, '04, above mentioned, it is clear that while the two nucleoli may be very

generally present in the late cleavage stages of the hybrid teleost embryo, the parental chromosomes are positively not separated into two groups. Thus during and after the third cleavage of the egg, the long and the short chromosomes of the two parents of the hybrid mingle together, the spindles never again showing any division into right and left sides with the corresponding separation of the chromosomes, according to the work of Häcker, so marked in the early divisions of the copepod egg. The double nucleolate condition, cannot, therefore, be taken as an indication of the autonomy of the paternal and maternal contributions.

In *Pedicellina*, moreover, the fertilization and early cleavage stages, where the autonomy of the parental chromatins would be most marked if at all present, is not at all in evidence. In spite of the bi-nucleolate condition of the later life history, it is clear from the early stages that the chromosomes of the egg and the sperm have, as in the Fundulus-Menidia hybrid of Moenkhaus, entirely mingled, perhaps in the first cleavage spindle. Thus, also in *Ascaris*, according to Zoja, '95, where the chromosomes of the egg and sperm have mingled as early as the 12-cell stage. In the Copepods themselves, it is not clear that the physiological distinction in the parental sides of the spindles persists after the early cleavages, and thus, the only important evidence for persistent gonomery is, in these types, the constant appearance of the two nucleoli, and this, it is clear, involves an assumption which, in view of the latest work, is full of difficulties. Of still less weight is the occurrence of bilobed nuclei in the late stages, these probably representing little more than an intermediate stage in the fusion of the several vesicles into one resting nucleus. The appearance of the double spirem in the early germ-cells while of greater value is, from the rarity of its occurrence and from the want of proof that the halves are parentally different, also indecisive.

It is therefore much more probable that the parental chromosomes actually mingle among themselves at an early period of development, and that these so-called outward expressions of internal independence are either accidental and unconnected with the chromatin phenomena or are expressive of other conditions

within the nucleus. By this it is not meant that the chromosomes lose their individuality or fuse into one indiscriminate mass. On the contrary, in most forms examined on this matter and in *Pedicellina* particularly, the persistence of this individuality is most marked although it is impossible to distinguish between the paternal and maternal chromosomes now mingled among themselves.

This conclusion is not only more in accordance with the facts but can alone directly explain the possibility of a parental synapsis, *i. e.*, the union of the homologous paternal and maternal chromosomes into pairs, thus reducing the number to one half at the close of the oögonic and spermatogonic cycles in the early germ-cells (Montgomery and Sutton). Were the homologous chromosomes segregated at this period also, this union would be impossible; mingled together, however, as Moenkhaus has actually shown in the teleost and as is very probable in other forms, the union would be readily effected.

What then is the significance of the two nucleoli so constant in the life history of *Pedicellina?* In the present state of our knowledge, when such uncertainty as to the nature and function of the nucleolus still persists, no definite answer can be given. In *Pedicellina* it is clear that they are not chromatin bodies and come only incidentally into connection with the chromosomes. They are plastin bodies in some way, very probably, connected with the waste products of division, as Häcker's theory maintains. Yet, whatever may be the significance of the nucleoli as organs of the cell, they do in some way reflect or represent the activities of the nuclear areas in which they arise. When, therefore, we find in the cells of the cleavage and later stages two nucleoli removed to opposite portions of the nucleus we may perhaps be permitted to infer that these are the outward expressions of the activities of these two portions which together compose the nucleus, and if an interpretation of parental nuclear autonomy be at all justified by the facts, and this is very questionable, I should suggest that it is much more probable that this autonomy applies to the paternal and maternal sap or ground substance in which the chromosomes are distributed.

In this connection, there is one more consideration to which attention must be drawn. In the spermatid of *Pedicellina*, the

chromatin appears in two masses; one localized at the anterior, the other at the posterior end of the now elongated sperm head, there being a non-staining middle space between (Fig. 11). On such a condition as this one might extend to this post-maturation stage the same interpretation that was criticized above. Indeed Häcker would so conclude. In his '02 work, referred to above, this author is quite ready to interpret the presence of two nucleoli in the matured sperm head as an indication of the persistent autonomy of the parental chromatin halves, even until this period. The only uncertainty, as he admits, in the Copepods under consideration (*Diaptomus* and *Heterocope*) being the presence of three or four nucleoli; nor are these placed at two opposite points of the nucleus as his hypothesis of gonomery demands.

FIG. 11.

In the matured egg, however, he finds absolutely no difficulty in accepting this conclusion. Speaking of the chromosomes at the beginning of the second maturation division, he states, p. 343: "Die 12 neuformirten bivalenten Elemente . . . werden durch den Reduktionsakt auf der zweiten Richtungskörper und Eizelle verteilt und letzterer enthält demnach 6 Mischlinge, welche sich je aus einer väterlichen und mütterlichen, oder, da die reife Eizelle bereits eine neue Generation repräsentiert, besser gesagt, aus einer groszväterlichen und groszmütterlichen Hälfte zusammensetzen." To understand fully what this post-maturation gonomery of Häcker involves, it is necessary to review in more detail the processes by which it is attained. In a late prophase of the first maturation mitosis, the twenty-four chromosomes (the somatic number) appear in two rows of six pairs each; the paired bivalent chromosomes having arisen through the union end to end of single chromosomes of the same parental side. Thus, the twelve paternal chromosomes, *a*, *b*, *c*, *d*, *e*, *f*, *g*, *h*, *i*, *j*, *k*, *l*, are arranged *ab*, *cd*, *ef*, *gh*, *ij*, *kl*, while correspondingly those of the maternal side as *no*, *pq*, *rs*, *tu*, *vw*, *xy*. Each of the pairs then splits longitudinally forming twelve tetrads, six completely paternal and six completely maternal. The first maturation which is equational reduces the tetrads to dyads leaving in the egg twelve bivalent chromosomes, six from each parental side. Now ensues a second synapsis between pairs, this time of

opposite parental sides, thus giving rise to six tetrads, viz; : $\frac{a}{n}\frac{b}{o}\,\frac{c}{p}\frac{d}{q}\,\frac{e}{r}\frac{f}{s}\,\frac{g}{t}\frac{h}{u}\,\frac{i}{v}\frac{j}{w}\,\frac{k}{x}\frac{l}{y}$, and through a subsequent rotation these become $\frac{a}{b}\frac{n}{o}\,\frac{c}{d}\frac{p}{q}\,\frac{e}{f}\frac{r}{s}\,\frac{g}{h}\frac{t}{u}\,\frac{i}{j}\frac{v}{w}\,\frac{k}{l}\frac{x}{y}$. The second or reducing division distributes these in such a manner that the egg and the second polar body each receives twelve chromosomes (six bivalents) of which six single elements are paternal and six maternal ; thus, *an*, *cp*, *er*, *gt*, *iv*, *kx*, go to the now matured egg while the rest go to the second polar body.

There is, however, but little probability for such an interpretation as it entirely ignores the significance of the primary synapsis which from the figures of Rückert, '04, and Häcker, '95, himself, undoubtedly takes place at the beginning of oöcytic growth. This undoubtedly results in the reduction of the chromosomes during this period. From the highly probably results of Montgomery and Sutten (which have received strong corroboration from all sides) the twelve bivalents of the first maturation prophase would represent the union of homologous paternal and maternal chromosomes and not, as Häcker assumes, of the same parental side, *i. e.*, paternal with paternal, maternal with maternal On the first of these hypotheses the two ensuing maturation divisions would produce conditions quite analogous to that observed by the great majority of workers on the germ-cells of both animals and plants. But what is far more convincing is the fact, that on the interpretation of Häcker, there would result, in the great majority of cases, an egg with such a chromatic constitution that the potentialities of one group of characters would often be doubled, while those of another group would be entirely lacking. This would not only make Mendelian results impossible but, as Boveri has shown in his work on "Multipolar-mitoses" might lead to the production of defective larvæ.

We must therefore conclude that the segregation of the chromatin in the spermatid of *Pedicellina* is without particular significance and that the gonomery or autonomy of the parental chromatic contributions continues at most only up to the beginning of the growth period of the germs-cells, the mingling having

occurred, in most cases, long before this. At this point, in the processes of synapsis the several chromosomes of the paternal side very probably individually unite with their correspondents on the maternal side. The position of the thus-formed bivalents "in the equatorial plate of the reducing division is purely a matter of chance, that is, that any chromosome pair may lie with maternal or paternal chromatid indifferently toward either pole irrespective of the position of other pairs and hence, that a large number of combinations of paternal and maternal chromosomes are possible in the matured germ products of an individual" (W. S. Sutton, "The Chromosomes in Heredity," BIOL. BULL., IV., 5).

II.

The nuclei of the several generations of oögonia have already been described (Fig. 3) and need not again be considered except to note that the two nucleoli or the one after the fusion are always homogenous in structure and stain exactly like plastin

FIG. 12.

bodies. These invariably disappear in the ensuing mitosis. In the youngest oöcytes, a new nucleolus, more rarely two, makes its appearance (Fig. 12, *a*). This body is then quite small and may be situated anywhere within the nucleus; sometimes near the periphery, sometimes on an arm of a chromosomal V or even in the midst of a number of such intersecting arms. It stains intensely at this stage, and shows as yet no trace of vacuolization. In iron-hæmatoxylin, carm-alum, Borel's fluid, etc., it stains more deeply than the chromatin itself and might, if superficially considered, be looked upon as a chromatic nucleolus, as several workers have, on such evidence as this, actually considered it. In Auerbach's fluid, however, the true nature of the body is very clearly brought out. It stains invariably a deep red with the acid fuchsin and shows no trace of the methyl green with which the chromosomes are colored.

As the oöcyte grows, the nucleolus increases in size, and at the same time usually becomes associated with one or more of the chromosomes, and about this period a small vacuole makes its appearance in the center of the body. It stains very slightly and stands out in marked contrast to the darker outer portion (Fig. 12, *b*). From this point onward the course in nucleolar changes is fairly direct and consists in the constant increase of the area of vacuolization until at the end of the period of growth only a minimal portion of the now very large nucleolus shows any stain.

This process of vacuolization may, however, take one of two courses. In the first case, there arise one or more small vacuoles in the immediate vicinity of the first (Fig. 12, *c*), and, with the growth of the nucleolus, the number of these also increases (Fig. 12, *d*), until finally a structure of honey-comb appearance is produced (Fig. 12, *e*). The vacuoles, which fill up the entire space of the nucleolus, are now fairly uniform in size. In the second case the additional vacuoles fuse with the primary one, or the latter may increase in size directly. In either instance, the end result is the same, viz., the central area is much increased in di-

FIG. 13.

ameter, and by the time the yolk makes its appearance in the egg-cytoplasm only a thin outer rim remains of the deeply-staining portion (Fig. 13, *a*, *b*). Vacuoles may, however, later make their appearance in this outer rim (Fig. 13, *c*), showing very clearly that there is no fundamental distinction between the two types above described.

In both these groups, moreover, additional nucleoli often arise secondarily, and these pass through the same processes described for the primary body. In some instances these may fuse, giving rise to a compound nucleolus, one part of which is generally, as is shown by the differences in staining reaction, a little further

advanced than the other (Fig. 13, *d*). In these types also there are often observed certain individual variations which are of interest in their bearing on the relations between the nucleolus and the chromosomes. A cap of deeply-staining material, showing no sign of vacuoles within it, often covers one part of the structure. This varies in size, from the merest rim to a mass half as large as the fully grown nucleolus itself. In some cases there are two such caps to the same nucleolus (Fig. 14, *a*). The other variation consists in the frequent indentation of the outer rim, which gives the appearance of disintegration on the part of the nucleolus (Fig. 14, *b*). This view is made probable by the presence in such cases of large granules, which lie within the indentation and seem directly to fit into it. These conditions, moreover, are found only in advanced stages of egg-development. Altogether, the appearance very strikingly resembles Fig. 13 of

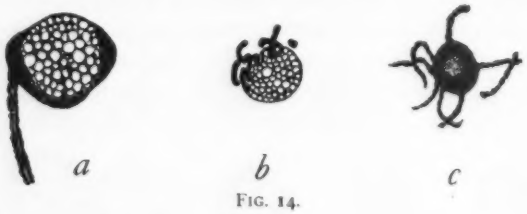


FIG. 14.

Guenther's work, '03, on the nucleolus in the maturing eggs of the Echinodermata (*Holothuria tubulosa*).

This author considers this one of the strongest elements in the evidence, for the chromatic nature of the nucleolus, since the indentation is taken to signify that the nucleolar substance has broken off to become a chromosome. Such a picture as that shown in Fig. 14, *b*, where, in an egg nearly ready for the first maturation, some of the much concentrated chromosomes actually lie within a nucleolar indentation, would most probably be interpreted by Gunther, '03, and some other authors, as very strong evidence in favor of their hypothesis. Indeed, when stained with hæmatoxylin, some of the chromosomes are in such intimate connection with the nucleolus, that all the necessary conditions which their hypothesis demands seem to be realized. Yet nothing can be further from the truth. I have fortunately found such a stage

among my Auerbach preparations, and the results dispel any possibility of confusion. The nucleolus, at this point completely vacuolated, stains intensely red and, in most striking contrast, all the chromosomes lying on its side are as intensely green. It is hardly possible that this striking distinction in staining capacity would exist if there were any more intimate connection between the chromatin and the nucleous, than mere apposition. But more conclusive evidence is given by the fact that the characteristic concentration and localization of the chromosomes, immediately before the first maturation division, is, in most instances, passed through without any relation to the nucleolus. In addition, the history of the chromatin in the growing oöcyte is of a nature entirely incompatable with the conception of the authors cited above. As is pointed out in the section on oögenesis (Dublin, '05), the chromosomes persist, in the reduced number, from the period of synapsis up to the time when they enter into the first maturation spindle. At no stage is there a break in this continuous history. At no stage is a chromatin reticulum formed which might, in some way, make the continued observations of the chromosomes an uncertain task. On this point, the evidence appears to be decisive. These indented nucleoli often occur, when the chromosomes are at the highest point of their extension, as threads, and when even the smallest of them would be many times as large as the space out of which they are supposed to arise. We can now understand the true significance of a condition such as is shown in Fig. 14, *c*, where the chromosomes radiate from the nucleolus and seem to originate from it. This is simply a case, rare enough, where the chromosomes in their extension through the nucleus have crossed such a body. It is probable, further, that they have been intimately associated with the nucleolus by the coagulation incident to the fixing process.

In connection with the deep staining cap-formation on the much vacuolated nucleolus, it is very probable that we are concerned with a phenomena similar in all respects to that observed by Obst, '99, in *Limax*, and in other Molluscs, where a nucleolus was found to arise from the chromatin in the process of concentration. These nucleoli arise late in the oöcytic growth and, in contrast with the cyanophilous nucleoli of the early oöcytes, they

are erythrophilous. In *Pedicellina*, at the stage of the formation of these caps, the chromosomes are in the process of shortening from long ragged threads to small thick rings and bars of the first maturation mitosis, and it is evident that a considerable part of their substance is lost during the change. Fig. 14, *a*, shows very clearly the apposition of one of these long chromosomes to the cap with the strong probability that the substance of the latter is being increased at the expense of that of the former.

As in *Limax* also, the nucleolus of the *Pedicellina* ovum plays no observable part in the formation of the first polar figure. Its structure, now much reduced, disintegrates and its remains are cast out into the light area with which the spindle is surrounded.

The facts observed appear to harmonize, in the main, with the general conclusions which Häcker, '95, has reached. At no period of egg-development is there any possibility for the origin of chromatin from the nucleoli. Indeed, the only perceptible relation which it may have with the chromosomes, excepting those obviously ascribable to accidental apposition, are those where the former adds to its own substance from the cleavage products of the latter. When the uncertain evidence of such works as those of Guenther and Hartman, and the absolute denial given by Häcker, '02, Miss King, '01, and Janssen, '04, to the results of Carnoy and LeBrun, '97–'99, is considered, then it may be concluded that the true nucleoli may, after all, be of one type, and are to be distinguished from the undoubted chromatin bodies such as those described by Blackman, '03, Wilson, '01, and others which are but temporary aggregations of chromatin unwinding the materials from which they are formed.

It gives me great pleasure to acknowledge my indebtedness to Prof. E. B. Wilson under whose observation this study was made.

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January 6, 1905.

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FOSSIL CEPHALOPODA, DESCRIBED BY HYATT AND CRAGIN, IN THE MUSEUM OF THE UNIVERSITY OF TEXAS.[1]

ANNIE H. PRITCHETT.

In the Second and Third Reports of the Geological Survey of Texas, published in 1890 and 1892 respectively, Professor Alpheus Hyatt described a number of Nautiloids from material collected in Texas under the direction of the then State Geologist, Mr. E. T. Dumble; and in the Fourth Report, Professor F. W. Cragin describes a number of forms, mainly Ammonites. The material described in these three papers represents only a portion, about an eighth, of a rather extensive collection of specimens of these groups, but the portion that is most interesting because of the number of type specimens. Only recently has this material been unpacked and arranged, and because it is the largest collection of the kind in Texas, and because for so many years it has been inaccessible to students, it seemed desirable to publish a short list of the more important specimens. The task has been relatively simple, since in only a few cases had the original labels been lost or misplaced; and in most cases the describers had marked new species with the word "type."

In the following list the name of each species is followed in parentheses by the name of the particular describer, and the volume and page of the published Report.

NAUTILOIDEA.

Temnocheilus conchiferous, type (Hyatt, Second Rep., p. 329).
T. forbesianus McChesney (Hyatt, Second Rep., p. 330).
Tainoceras cavatum, type (Hyatt, Second Rep., p. 341).
T. quadrangulum McChesney (Hyatt, Fourth Rep., p. 402).
Stearoceras gibbosum, type (Hyatt, Fourth Rep., p. 424).
Coloceras globulare, type (Hyatt, Fourth Rep., p. 452).
Glyphioceras cumminsi, type (Hyatt, Fourth Rep., p. 467).
G. incisum, type (Hyatt, Fourth Rep., p. 471).

[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 66.

Phacoceras dumbli, type (Hyatt, Second Rep., p. 347).

Gastrioceras compressum, type (Hyatt, Second Rep., p. 355).

G. entogonum Gabb (Hyatt, Fourth Rep., p. 472).

Metacoceras walcotti, type (Hyatt, Second Rep., p. 337).

M. inconspicuum, type (Hyatt, Second Rep., p. 340).

Domatoceras militarium, type (Hyatt, Fourth Rep., p. 445).

(The type specimen of *Domatoceras simplex* Hyatt, Fourth Rep., p. 441, appears to have been lost.)

Ephippioceras divisum White and St. John (Hyatt, Second Rep., p. 350).

Solenocheilus collectus Meek and Worthen (Hyatt, Fourth Rep., p. 463).

Paralegoceras iowense Meek and Worthen (Hyatt, Fourth Rep., p. 474).

Nautilus texanus Shumard (Cragin, Fourth Rep., p. 236).

AMMONITOIDEA.

Placenticeras syrtalis cumminsi, type (Cragin, Fourth Rep., p. 237).

Pulchella bentonianum, type (Cragin, Fourth Rep., p. 239).

Scaphites septum-seriatus, type (Cragin, Fourth Rep., p. 240).

Sphenodiscus dumbli, type (Cragin, Fourth Rep., p. 243).

S. rœmeri, type (Cragin, Fourth Rep., p. 245).

(The type specimen of *Sphenodiscus emarginatus*, Cragin, Fourth Rep., p. 245, appears to have been lost.)

S. lenticularis Owen (Cragin, Fourth Rep., p. 245).

Hoplites rœmeri, type (Cragin, Fourth Rep., p. 234).

H. texanus, type (Cragin, Fourth Rep., p. 235).

Pachydiscus brazoensis Shumard (Cragin, Fourth Rep. p. 236).

P. complexus Hall and Meek (Cragin, Fourth Rep., p. 237).

Crioceras annulatus Shumard (Cragin, Fourth Rep., p. 234).

Schlœnbachia peruviana Von Buch (Cragin, Fourth Rep., p. 242).

S. woolgari Mantell (Cragin, Fourth Rep., p. 243).

S. leonensis anatina Conrad (Cragin, Fourth Rep., p. 241).

Buchiceras inœquiplicatus Shumard (Cragin, Fourth Rep., p. 233).

B swallowi Shumard (Cragin, Fourth Rep., p. 234).

UNIVERSITY OF TEXAS,
January 15, 1905.